A recognition-mediated reaction drives amplification within a dynamic library

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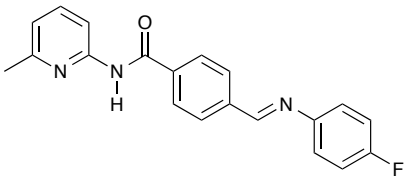
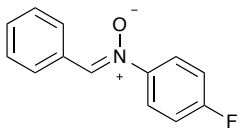
Electronic Supplementary Information

Compound preparation and spectroscopic data



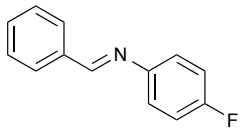
(*E*)-4-((4-fluorophenylimino)methyl)-*N*-(6-methylpyridin-2-yl)benzamide 1

N-(6-methyl-2 pyridyl)-4-formyl benzamide (0.50 g, 2.1 mmol) and 4-fluoroaniline (0.23 g, 2.1 mmol) were dissolved in minimal amount of ethanol and left in the absence of light for 16 h. The resulting precipitate was filtered affording the desired compound as a yellow solid (0.52 g, 76%). M.p.=154.8-155.4°C; ^{1}H NMR (400.1 MHz, $CDCl_3$, 25°C, TMS): δ=8.62 (s, 1H; NH), 8.54 (s, 1H; CH), 8.22 (d, ^{3}J(H,H)=8 Hz, 1H; ArH); 8.07-8.02 (m, 4H; ArH), 7.69 (t, ^{3}J(H,H)=8 Hz, 1H; ArH), 7.29-7.25 (m, 2H; ArH), 7.16-7.10 (m, 2H; ArH), 6.90 (d, ^{3}J(H,H)=8 Hz, 1H; ArH), 2.50 (s, 3H; CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ=164.9 (CO), 163.2 (ArC), 160.0 (ArC), 158.6 (CH), 157.0 (ArC), 150.6 (ArC), 139.6 (ArC), 138.9 (ArCH), 136.6 (ArC), 129.1 (ArCH), 127.7 (ArCH), 122.5 (d, ^{3}J(C,F)=8 Hz; ArCH), 119.7 (ArCH), 116.0, (d, ^{3}J(C,F)=23 Hz; ArCH) 111.1 (ArCH), 24.0 (CH_3); ^{19}F NMR (376.5 MHz, $CDCl_3$, 25°C): δ=–116.7 (ArF); MS (ES+): *m/z* (%): 356.04 (100) [M^++Na]; HRMS (ES+) calculated for $C_{20}H_{16}N_3OFNa$ [M^++Na] 356.1174, found 356.1175.



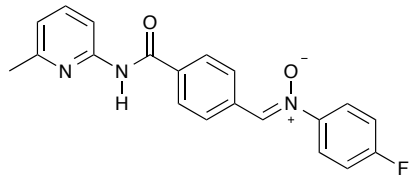
(*E*)-*N*-benzylidene-4-fluoroaniline oxide 2

Benzaldehyde (0.5 g, 4.7 mmol) and *N*-(4-fluorophenyl)hydroxylamine (0.6 g, 4.7 mmol) were dissolved in minimal amount of ethanol and left in the absence of light for 16 h. The resulting precipitate was filtered affording the desired compound as a colourless crystalline solid (0.88 g, 88%). M.p.=169.5-170.3°C; ^{1}H NMR (400.1 MHz, $CDCl_3$, 25°C, TMS): δ=8.31-8.21 (m, 2H; ArH), 7.80 (s, 1H; CH), 7.71 (d, ^{3}J(H,H)=9 Hz, 2H; ArH), 7.41-7.39 (m, 3H; ArH), 7.17 (d, ^{3}J(H,H)=9 Hz, 2H; ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ= 164.8 (ArC), 161.4 (ArC), 134.6 (CH), 131.1 (ArCH), 130.6 (ArC), 129.1 (ArCH), 128.7 (ArCH), 123.7 (d, ^{3}J(C,F)=9 Hz; ArCH), 116.1 (d, ^{3}J(C,F)=23 Hz; ArCH); ^{19}F NMR (376.5 MHz, $CDCl_3$, 25°C): δ=–110.4; MS (ES+): *m/z* (%): 237.97 (100) [M^++Na], 453.04 (20); HRMS (ES+): calculated for $C_{13}H_{10}NOFNa$ [M^++Na] 238.0647, found 238.0644.



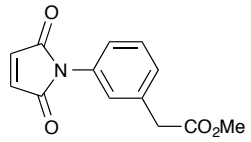
(*E*)-*N*-benzylidene-4-fluoroaniline 3

Benzaldehyde (0.50 g, 4.7 mmol) and 4-fluoroaniline (0.52 g, 4.7 mmol) were dissolved in minimal amount of ethanol and left in the absence of light for 16 h. The resulting precipitate was filtered affording the desired compound as a colourless crystalline solid (0.92 g, 98%). M.p.=56.0-57.0°C; ^{1}H NMR (400.1 MHz, $CDCl_3$, 25°C, TMS): δ=8.47 (s, 1H; CH), 7.95-7.92 (m, 2H; ArH), 7.50-7.48 (m, 3H; ArH), 7.25 (d, ^{3}J(H,H)=9 Hz, 2H; ArH) 7.12 (d, ^{3}J(H,H)=9 Hz, 2H; ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ=162.9 (ArC), 160.2 (CH), 159.7 (ArC), 136.1 (ArC), 131.5 (ArCH), 128.9 (ArCH), 128.8 (ArCH), 122.3 (d, ^{3}J(C,F)=8 Hz; ArCH), 115.9 (d, ^{3}J(C,F)=23 Hz; ArCH); ^{19}F NMR (376.5 MHz, $CDCl_3$, 25°C): δ=-117.8; MS (ES+): *m/z* (%): 199.99 (100) [M+H^{+}]; HRMS (ES+): calculated for $C_{13}H_{11}NF$ [M+H^{+}] 200.0874 found 200.0876.



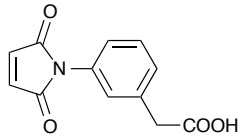
(*Z*)-4-fluoro-*N*-(4-(6-methylpyridin-2-ylcarbamoyl)benzylidine)aniline oxide 4

N-(6-methyl-2-pyridyl)-4-formyl benzamide (0.57 g, 2.4 mmol) and *N*-(4-fluorophenyl)hydroxylamine (0.30 g, 2.4 mmol) were dissolved together in ethanol (10 mL) and left in the absence of light to react for 16 h. The resulting precipitate was filtered and washed with cold ethanol to yield the desired product as a colourless solid (0.54 g, 64%). M.p.= 217.2-218.3°C; ^{1}H NMR (400.1 MHz, $CDCl_3$, 25°C, TMS): δ=8.70 (s, 1H; NH), 8.37 (d, ^{3}J(H,H)=8 Hz, 2H; ArH), 8.12 (d, ^{3}J(H,H)=8 Hz, 1H; ArH); 7.96 (d, ^{3}J(H,H)=8 Hz, 2H; ArH), 7.54 (s, 1H; CH), 7.72 (d, ^{3}J(H,H)=8 Hz, 2H; ArH), 7.58 (t, ^{3}J(H,H)=8 Hz, 1H; ArH), 7.08 (d, ^{3}J(H,H)=8 Hz, 2H; ArH), 6.87 (d, ^{3}J(H,H)=8 Hz, 1H; ArH), 2.36 (s, 3H; CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ=165.2 (CO),165.0 (ArC), 161.6 (ArC), 157.0 (ArC), 138.8 (ArCH), 135.8 (ArC), 133.7 (ArC), 133.3 (CH), 129.0 (ArC),127.6 (ArC), 123.7 (d, ^{3}J(C,F)=9 Hz; ArCH), 119.7 (ArCH), 116.2 (d, ^{3}J(C,F)=23 Hz; ArCH), 111.1 (ArCH), 24.0 (CH_3); ^{19}F NMR (376.5 MHz, $CDCl_3$, 25°C): δ=-109.6 (ArF); MS (ES+): m/z (%): 372.2 (100) [M^{+}+Na]; HRMS (ES+): calculated for $C_{20}H_{16}N_3O_2FNa$ [M^{+}+Na] 372.1124, found 372.1114.



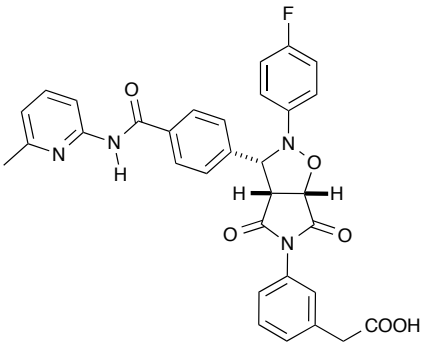
Methyl 2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)acetate 5a

2-(3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)phenyl)acetic acid **5b** (1.00 g, 4.3 mmol) was added to a solution of $NaHSO_4 \cdot SiO_2$ (100 mg/mmol) in methanol (20 mL) and left to stir under nitrogen atmosphere for 30 h. The suspension was filtered to separate the catalyst and filtrate was evaporated under reduced pressure to yield a yellow residue. The crude product was purified by silica gel flash column chromatography (DCM) affording a yellow solid (0.54 g, 51%). M.p.=58.3-59.0°C; ^{1}H NMR (400.3 MHz, $CDCl_3$, 25°C, TMS): δ=7.45-7.41 (m, 1H; ArH), 7.31-7.25 (m, 3H; ArH), 6.85 (s, 2H; 2xCH), 3.70 (s, 3H, CH_3), 3.67 (s, 2H, CH_2); ^{13}C NMR (125.7 MHz, $CDCl_3$, 25°C): δ=171.6 (CO), 169.5 (CO), 135.2 (CH), 134.4 (ArC), 131.5 (ArC), 129.4 (ArCH), 129.0 (ArCH), 127.0 (ArCH), 124.9 (ArCH), 52.3 (CH_3), 41.0 (CH_2); MS (ES+): m/z (%): 268.0 (100) [M^++Na] ; HRMS (CI+): calculated for $C_{13}H_{12}NO_4$ [M^++H] 246.0766, found 246.0771.



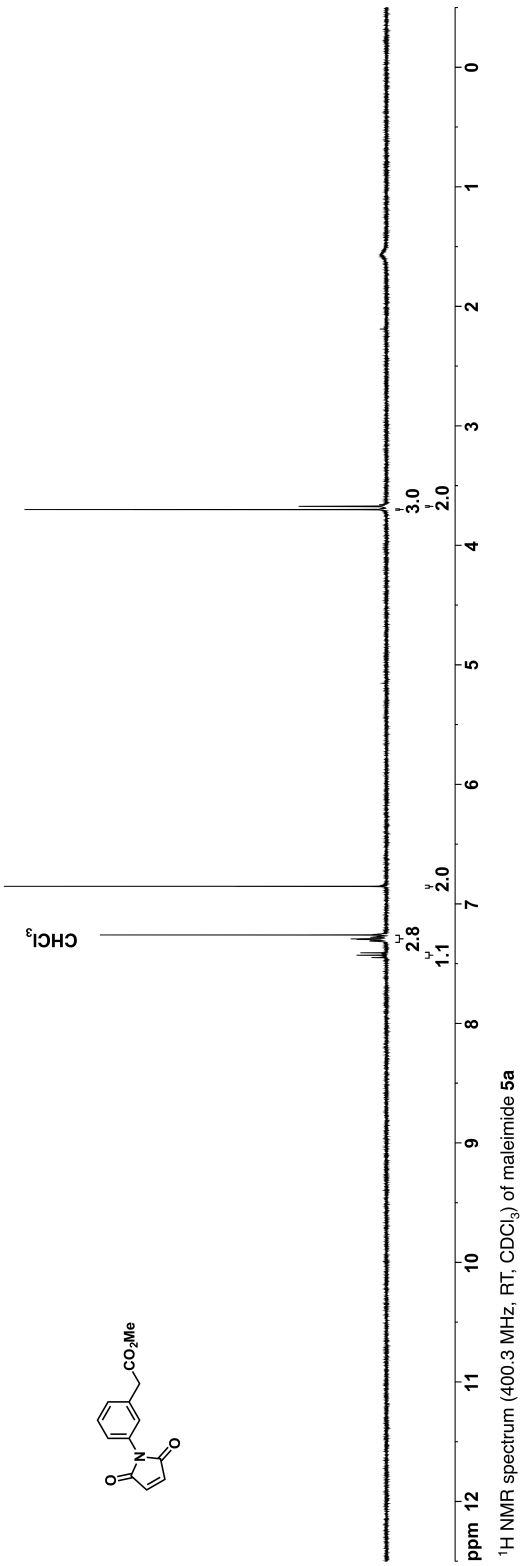
2-(3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)phenyl)acetic acid 5b

3-aminophenylacetic acid (5.00 g, 33.1 mmol) and maleic anhydride (3.20 g, 32.7 mmol) was dissolved in glacial acetic acid (200 mL). The solution was stirred at room temperature under nitrogen for 12 h, followed by heating to reflux for further 5 h. The solvent was removed *in vacuo* and the residue was purified on silicagel chromatography column (hexane:ethyl acetate, 3:1) affording the desired product as a yellow solid which was recrystalized from DCM (3.53 g, 46%). M.p.=114.3-115.0°C; ^{1}H NMR (300.1 MHz, [D_6]DMSO, 25°C, TMS): δ=12.44 (s, 1H; COOH), 7.46-7.40 (m, 1H; ArH), 7.30 (dt, ^3J(H,H)=7.8 and 1.4 Hz, 1H; ArH), 7.23-7.20 (m, 2H; ArH), 7.18 (s, 2H, 2xCH), 3.63 (s, 2H, CH_2); ^{13}C NMR (75.5 MHz, [D_6]DMSO, 25°C): δ=172.4 (CO), 170.0 (CO), 135.9 (ArC), 134.7 (CH), 131.5 (ArC), 129.0 (ArCH), 128.8 (ArCH), 127.7 (ArCH), 125.2 (ArCH), 40.4 (CH_2); MS (CI+): *m/z* (%): 231.0 (15) [M^+], 186.1 (100), 214 (15); HRMS (CI+): calculated for $C_{12}H_9NO_4$ [M^+] 231.0532, found 231.0540.

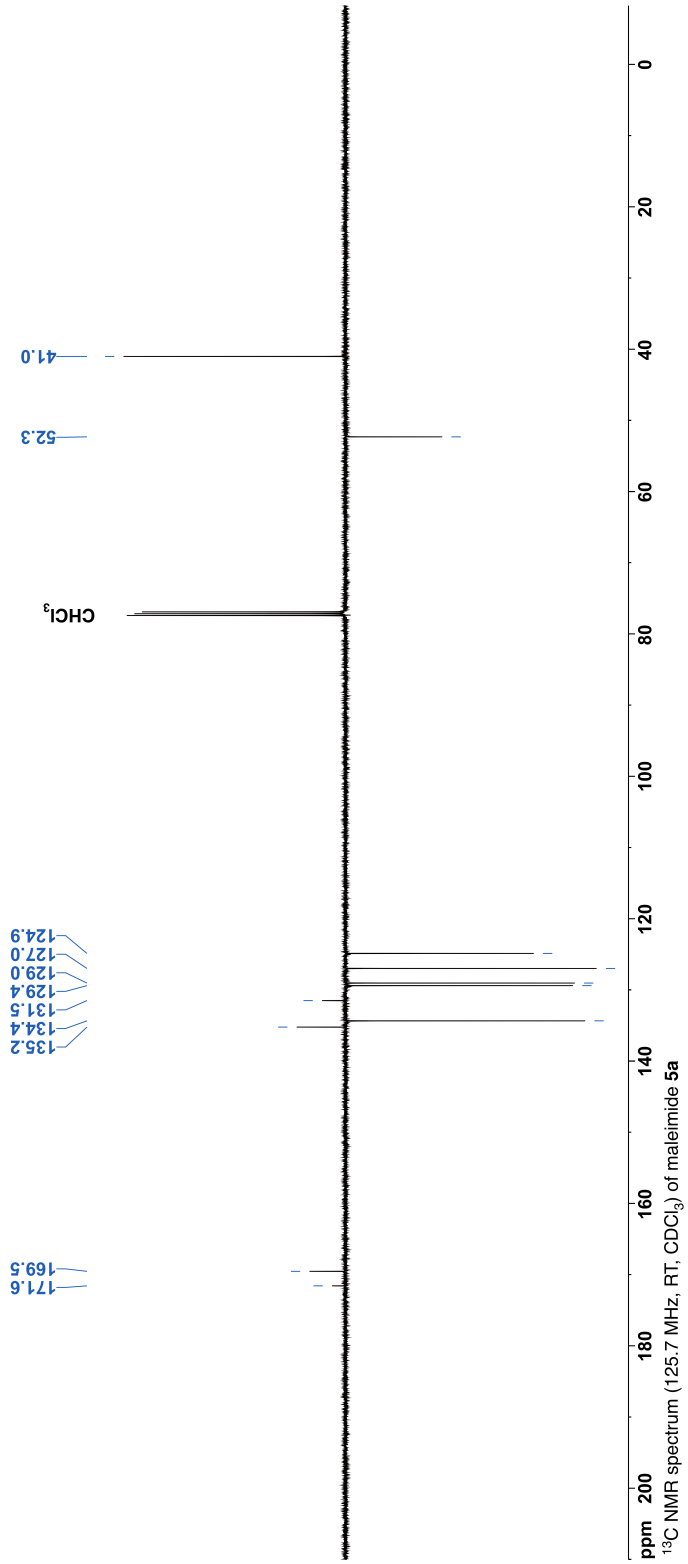


2-(3-(2-(4-fluorophenyl)-3-(4-(6-methylpyridin-2-ylcarbamoyl)phenyl)-4,6-dioxodihydro-2*H*-pyrrolo[3,4-*d*]isoxazol-5(3*H*,6*H*,6a*H*)-yl)phenyl)acetic acid *cis*-7b

2-(3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)phenyl)acetic acid **5b** (0.13 g, 0.6 mmol) and (*Z*)-4-fluoro-*N*-(4-(6-methylpyridin-2-ylcarbamoyl)benzylidine)aniline oxide **4** (0.20 g, 0.6 mmol) were dissolved in chloroform (10 mL) and left at 0°C for 16 h. The resulting precipitate was filtered and recrystalised from ethanol affording the desired product as a colourless solid (0.30 g, 90%, contains <4% *trans*-**7b**). M.p.=214.0-214.9°C; ^{1}H NMR (300.1 MHz, $CDCl_3$, 25°C, TMS): δ=11.30 (s, 1H; NH), 8.25 (d, ^{3}J(H,H)=8.3 Hz, 1H; ArH), 8.03-7.99 (m, 2H; ArH), 7.74 (dd, ^{3}J(H,H)=8.3 and 7.6 Hz, 1H; ArH), 7.64-7.61 (m, 2H; ArH), 7.51 (t, ^{3}J(H,H)=1.6 Hz, 1H; ArH), 7.36 (t, ^{3}J(H,H)=7.8 Hz, 1H; ArH), 7.23 (ddd, ^{3}J(H,H)=8.1, 2.0 and 1.2 Hz, 1H; ArH), 7.15 (ddd, ^{3}J(H,H)=7.6, 1.6 and 1.0 Hz, 1H; ArH), 7.11-7.06 (m, 2H; ArH), 6.97-6.91 (m, 3H), 5.30 (d, ^{3}J(H,H)=7.7 Hz, 1H; CH), 4.91 (d, ^{3}J(H,H)=9.7 Hz, 1H; CH), 4.12 (dd, ^{3}J(H,H)=9.8 Hz and 7.7 Hz, 1H; CH), 3.84-3.71 (m, 2H; CH_2), 2.49 (s, 3H; CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25°C): δ=176.3 (CO), 173.8 (CO), 171.1 (CO), 167.2 (CO), 160.2 (d, ^{1}J(C,F)=244.5 Hz; ArCF), 155.9 (ArC), 151.8 (ArC), 143.0 (d, ^{4}J(C,F)=2.6 Hz; ArC), 140.4 (ArCH), 138.6 (ArC), 135.7 (ArC), 135.2 (ArC), 131.4 (ArC), 130.0 (ArCH), 129.5 (ArCH), 129.0 (ArCH), 127.2 (ArCH), 126.5 (ArCH), 124.0 (ArCH), 120.8 (d, ^{3}J(C,F)=8.2 Hz; ArCH), 119.9 (ArCH), 115.8 (d, ^{2}J(C,F)=22.6 Hz; ArCH), 113.2 (ArCH), 77.3 (CH), 70.7 (CH), 54.8 (CH), 38.9 (CH_2), 22.2 (CH_3); ^{19}F NMR (282.4 MHz, $CDCl_3$, 25°C): δ=-117.7 (ArF); MS (ES+): *m/z* (%): 603.24 (100) [M^++Na]; HRMS (ES+): calculated for $C_{32}H_{25}N_4O_6NaF$ [M^++Na] 603.1643, found 603.1656.

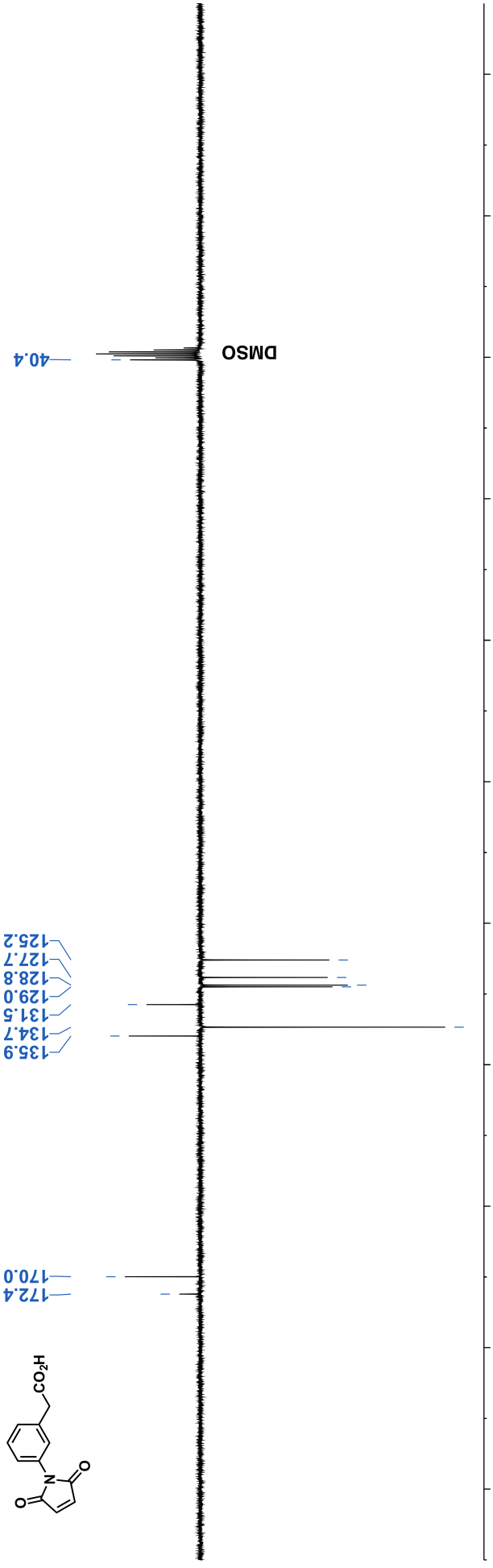


^{1}H NMR spectrum (400.3 MHz, RT, $CDCl_3$) of maleimide **5a**

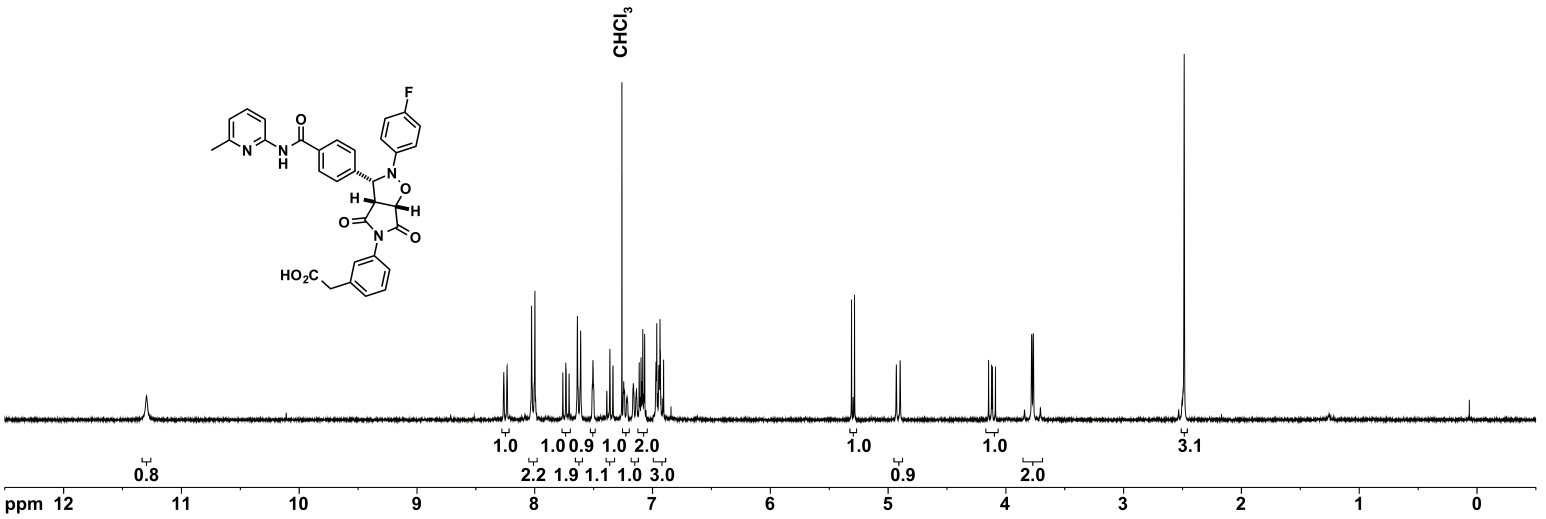


^{13}C NMR spectrum (125.7 MHz, RT, $CDCl_3$) of maleimide **5a**

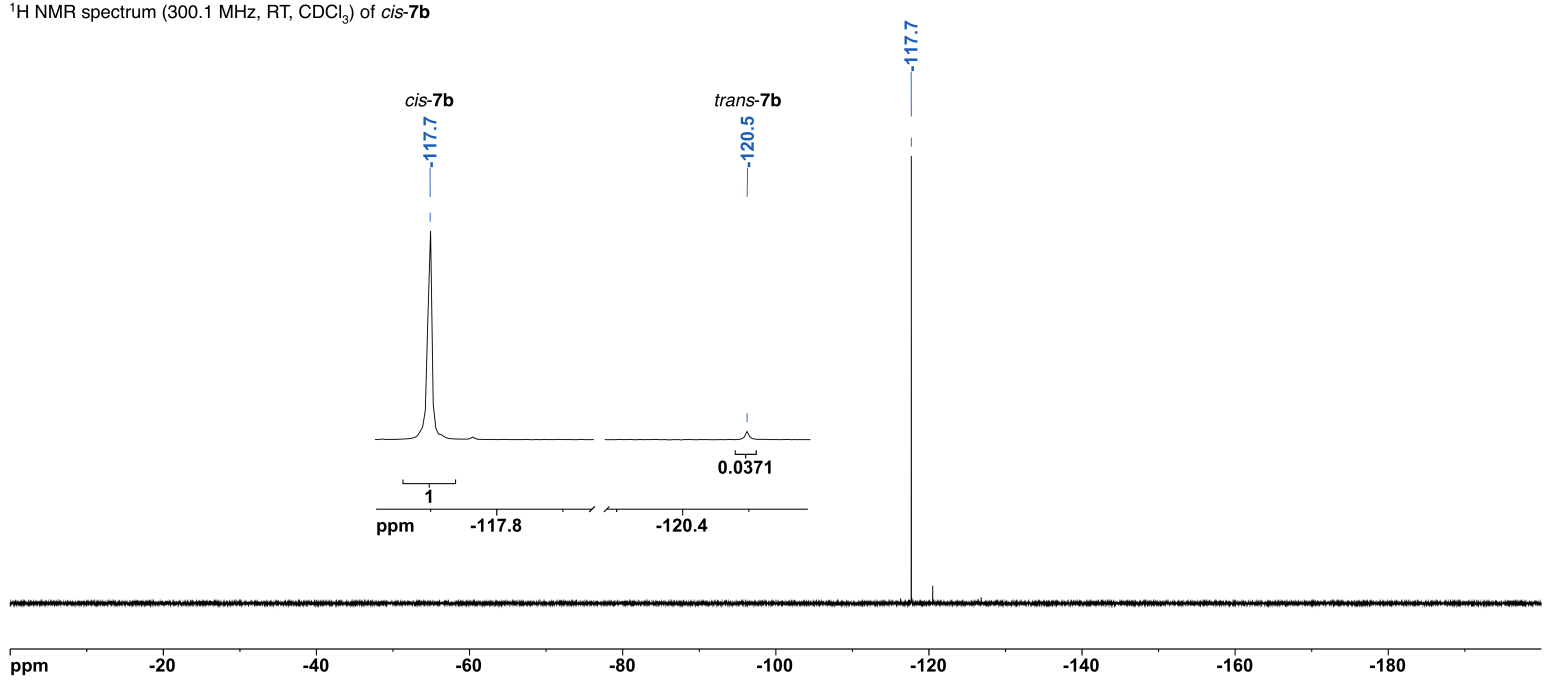
^{1}H NMR spectrum (300.1 MHz, RT, DMSO-d_6) of maleimide **5b**



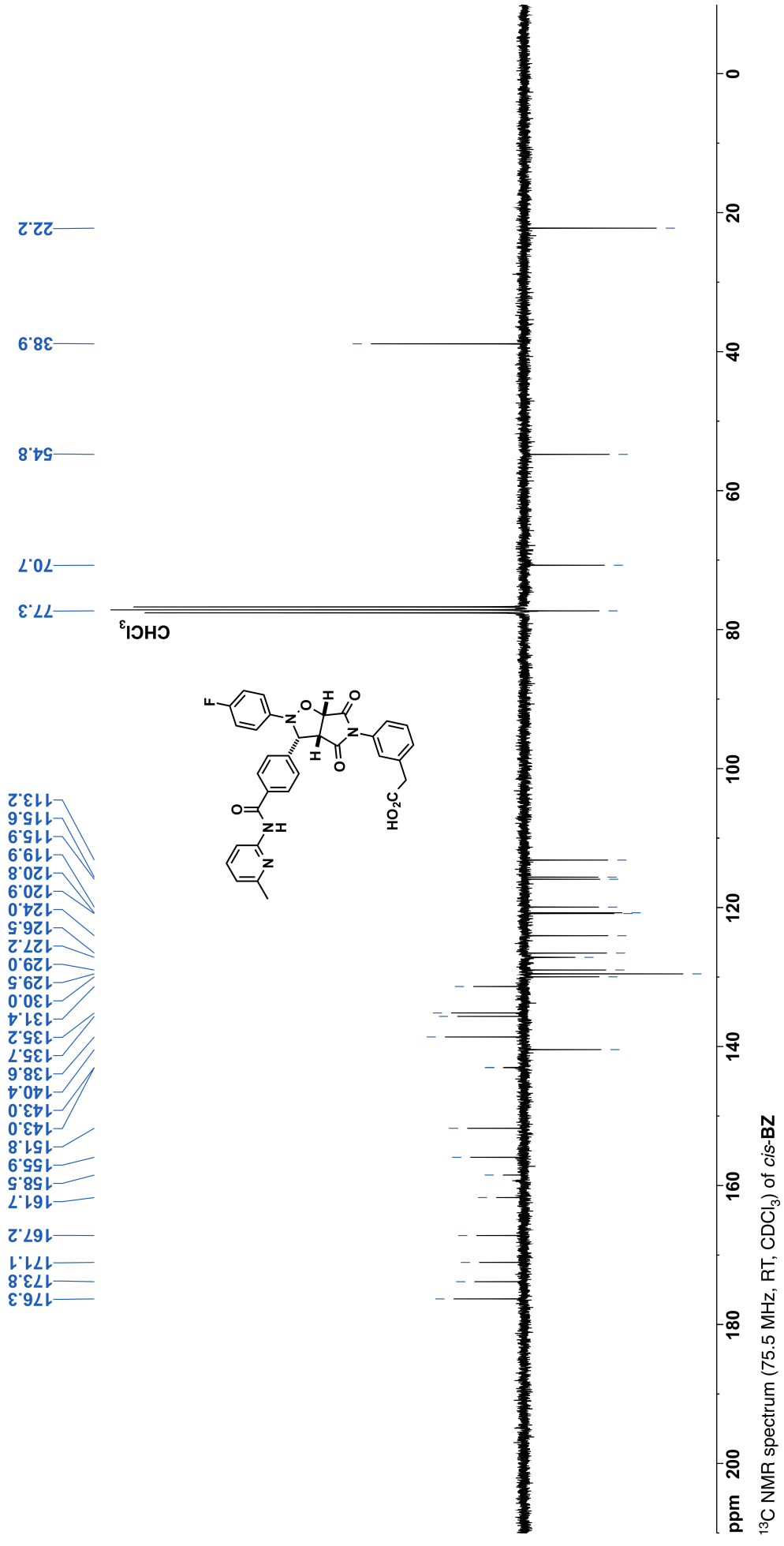
^{13}C NMR spectrum (75.5 MHz, RT, DMSO-d_6) of maleimide **5b**



^{1}H NMR spectrum (300.1 MHz, RT, $CDCl_3$) of *cis*-**7b**



^{19}F NMR spectrum (282.4 MHz, RT, $CDCl_3$) of *cis*-**7b** (contains <4% of *trans*-**7b**)



^{13}C NMR spectrum (75.5 MHz, RT, $CDCl_3$) of *cis*-**BZ**

Kinetic studies of the recognition-mediated reaction:

In order to probe that nitrone **4** and maleimide **5b** were capable of participating in a recognition-mediated reaction, we turned to electronic structure calculations. We located transition state structures linking the binary complex [**4•5b**] with cycloadduct *cis*-**7b** at the B3LYP/6-31G+(d,p) level of theory. Electronic structure calculations were carried out using GAMESS running on a Linux cluster. The 64-bit Linux version dated 1 May 2012 (Revision 2) was used in all calculations. The transition state for the reaction between **4** and **5b** was located by generation of an initial guess using the linear synchronous transit (LST) method and then refinement at the HF/6-31G(d) level of theory within GAMESS. This transition state guess was then refined at the B3LYP/6-31G+(d,p) level of theory to a transition state structure (**Figure S1**) possessing single imaginary vibration that corresponded to the reaction coordinate.

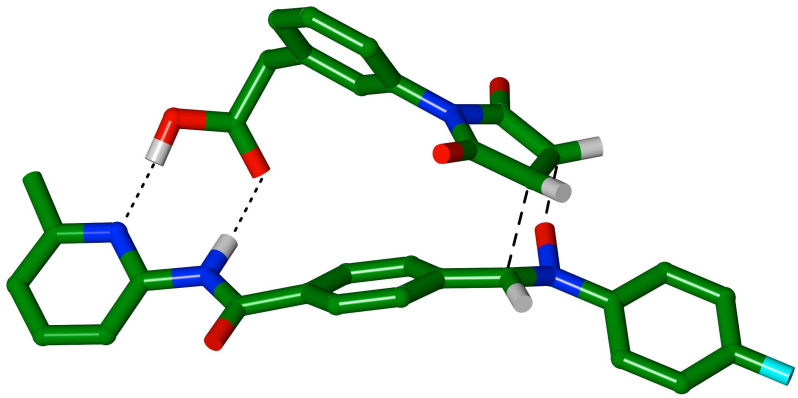


Figure S1 Calculated transition state (B3LYP/6-31G+(d,p)) for the reaction between nitrone **4** and maleimide **5b** within the [**4•5b**] binary complex. This transition state leads to *cis*-**7b**. C atoms are colored green, O atoms are colored red, N atoms are colored blue, H atoms are colored white and the F atom is colored cyan. Most H atoms have been omitted for clarity. Hydrogen bonds are represented by dotted lines and partial covalent bonds by dashed lines.

In order to verify experimentally the predicted behavior of the [**4•5b**] binary complex, we assessed the reactions of recognition-enabled nitrone **4** with both the control maleimide **5a** and the recognition-enabled maleimide **5b** (**Figure S2**)**.** These reactions were performed in CD_2Cl_2 at 0 ºC with the initial concentrations of the substrates of 25 mM and were monitored by 470.4 MHz ^{19}F NMR spectroscopy. The control reaction (**Figure S2**), using the methyl ester **5a**, exhibits initial rates for the formation of ***trans*-7a** of 3.5×10^{-5} mM s^{-1} and ***cis*-7a** of 1×10^{-5} mM s^{-1}. After 60000 s the total yield was 8 % and the ***trans*-7a**:***cis*-7a** ratio was 3.5:1. When the same experiment was conducted with the recognition motif enabled (**Figure S2**) the initial rate of the formation of the ***trans*-7b** was calculated to be 1×10^{-5} mM s^{-1} and the rate of formation of the ***cis*-7b** to be 1.25×10^{-3} mM s^{-1} – giving a rate acceleration for the formation of *cis*-**7b** of 125 ×. The conversion after 60000 s rose to 97 % and the diastereoisomeric ratio was 35:1 in favor of ***cis*-7b**.

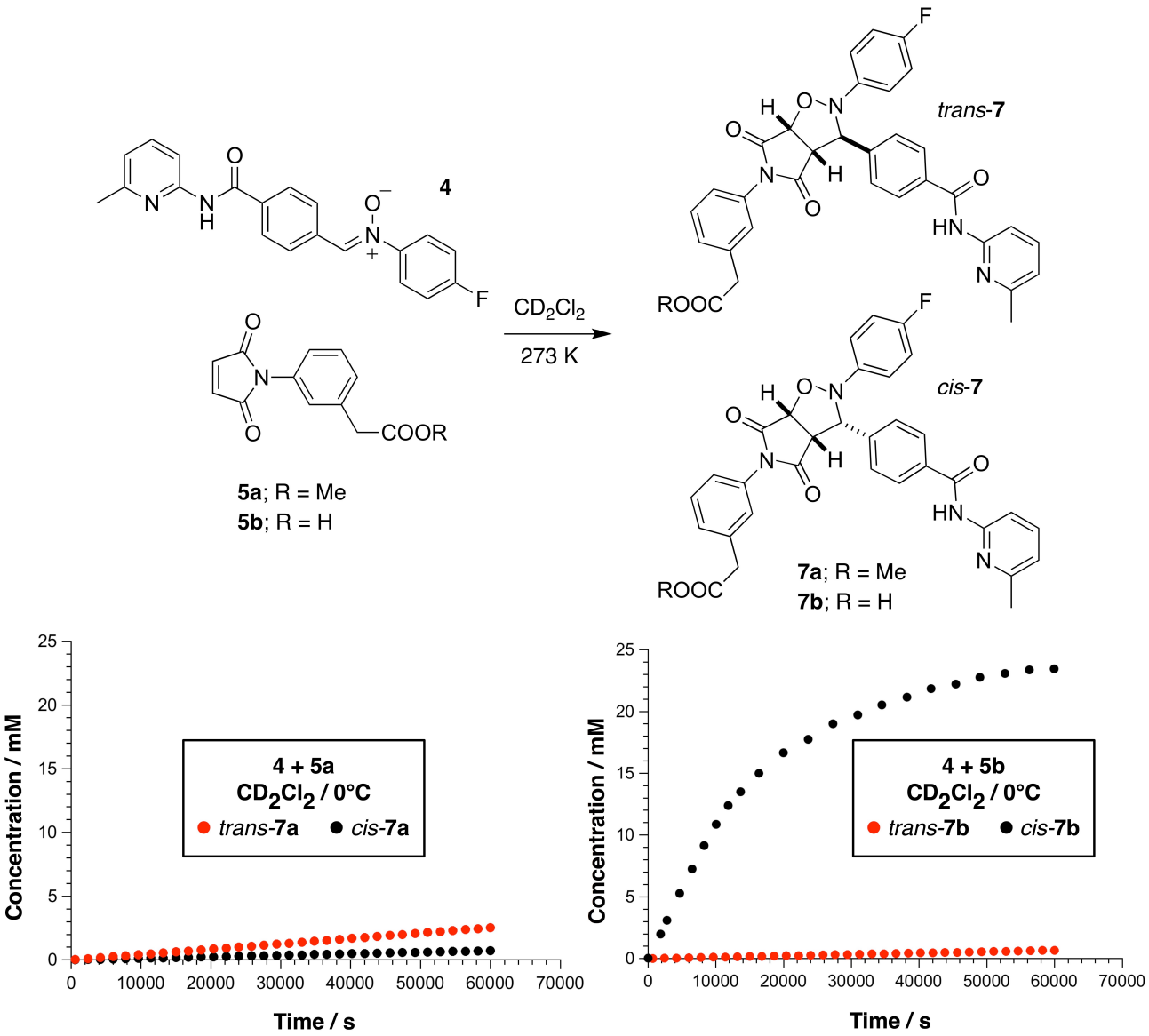


Figure S2 Concentration vs. time plots of the formation of ***trans*-7b** (black circles) and ***cis*-7b** (red circles) for the reactions between nitrone **4** and maleimide **5a** (left plot) and between nitrone **4** and maleimide **5b** (right plot).

Solvent Pretreatment

An important issue in the design of exchange experiments is the choice of solvent, since the exchange is influenced by the amount of water and acid in the medium. Drying and deacidifing a solvent such as $CDCl_3$ reproducibly proved problematic. In order to achieve reproducible conditions for the exchange process, CD_2Cl_2 was saturated with *p*-toluene sulfonic acid monohydrate (PTSA), supplying comparable quantities of water and acid into solution. Thus, CD_2Cl_2 (2 mL) was stirred rapidly with 100 mg of PTSA monohydrate in a volumetric flask at room temperature for 20 min. The suspension was filtered and the solvent used immediately.

General procedure for the preparation of exchange experiments:

Stock solutions of the appropriate nitrone, imine and maleimide were made using freshly prepared solvent and equilibrated in a thermostatically-controlled water bath at 273 K. In a typical experiment, an NMR sample was prepared in a 5 mm NMR tube (Wilmad 528PP) by mixing appropriate volumes of stock solutions such that the total volume was 0.7 mL and the concentration of each component equaled 20 mM. The NMR tube was transferred to an NMR spectrometer (Bruker Avance), regulated at 273 K, and 500.1 MHz ^{1}H and 470.4 MHz $^{19}F\{^{1}H\}$ NMR spectra were acquired automatically every 1800 s over a period of 16 hours. Analysis and deconvolution of each of the ^{1}H and ^{19}F NMR spectra recorded during this time was performed using Bruker Topspin software (Version 2.0 pl 3, Bruker Biospin, Germany, 2006). The data acquired from deconvolution was used to determine the concentrations of the mixture components at different time points. The compounds in the mixture were identified by their ^{19}F chemical shifts at 273 K in CD_2Cl_2 saturated with PTSA monohydrate. **Figure S3** shows typical 470.4 MHz ^{19}F NMR spectra recorded for the control and recognition-enabled exchange experiments. **Figure S4** shows each of the fluorine-containing compounds present in the exchanging mixture and their ^{19}F chemical shifts. **Figures S5** and **S6** show the concentration-time profiles for the control and recognition-enabled experiments respectively.

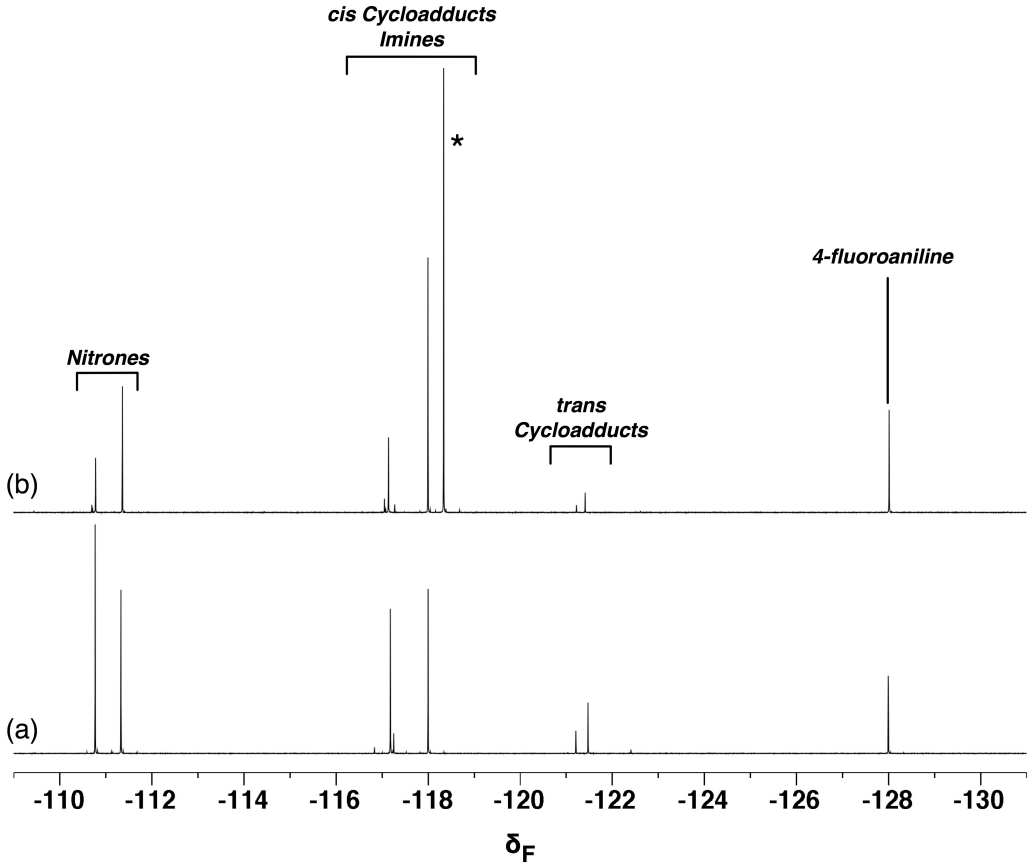


Figure S3 Partial 470.4 MHz ^{19}F NMR spectra, recorded in CD_2Cl_2 saturated with PTSA at 273 K, of (a) the control exchange experiment, starting from **1**, **2** and **5a**, after 16 hours and (b) the recognition-mediated exchange experiment, starting from **1**, **2** and **5b**, after 16 hours. The starred resonance is that arising from *cis*-**7b**.

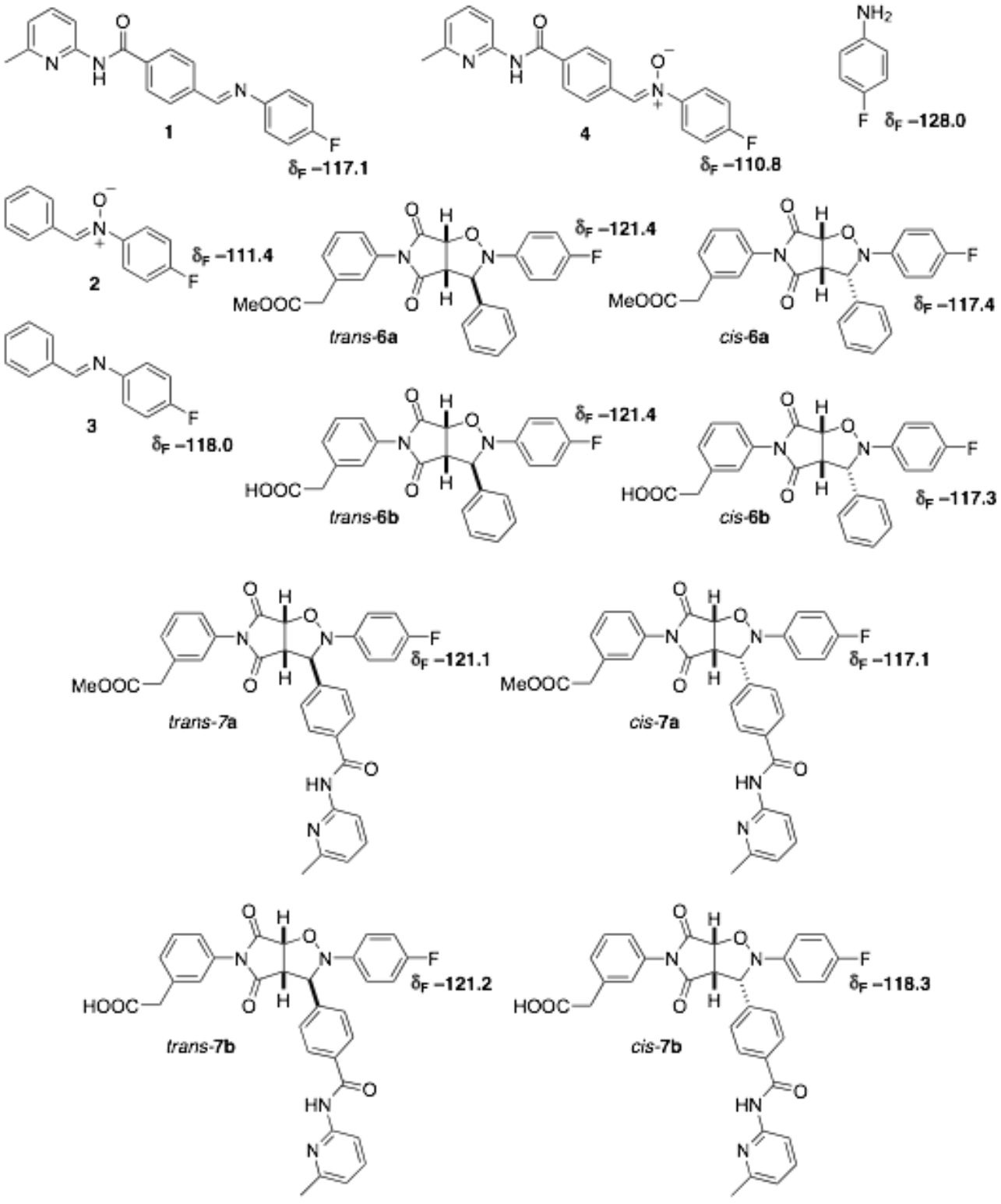


Figure S4 ^{19}F NMR chemical shifts, recorded in CD_2Cl_2 saturated with PTSA at 273 K, for all of the fluorine-containing species observed in the exchange experiments.

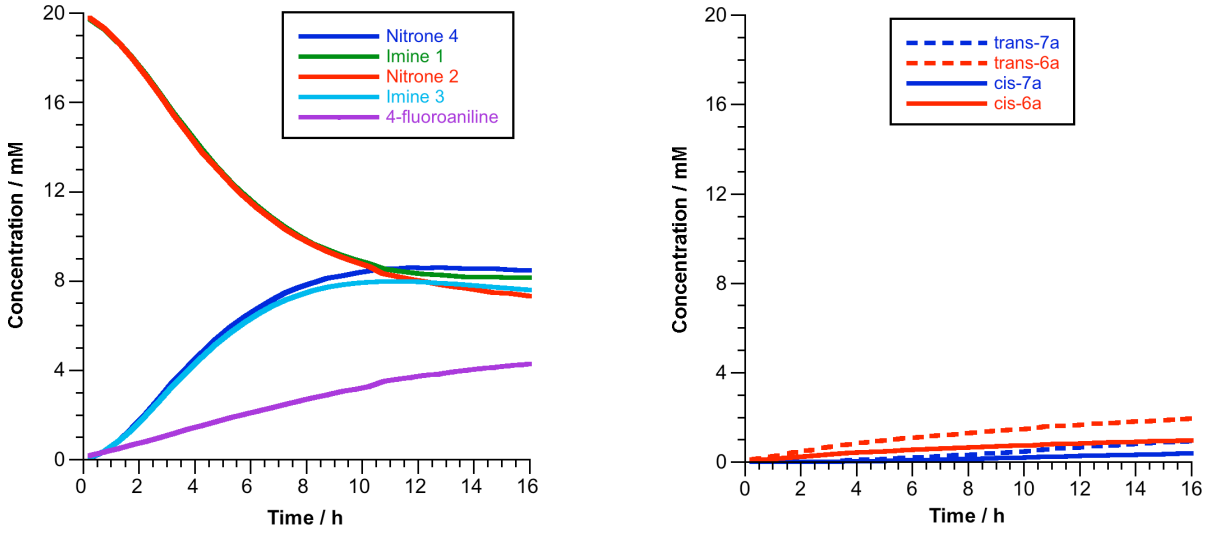


Figure S5 Concentration *vs.* time plot of the exchange process between nitrone **1** and imine **2** in the presence of the recognition-disabled maleimide **5a**. Conditions: [**1**] = [**2**] = [**5a**] = 20 mM; CD_2Cl_2 saturated with PTSA; 273 K. Concentration data derived from 470.4 MHz ^{19}F NMR spectroscopy.

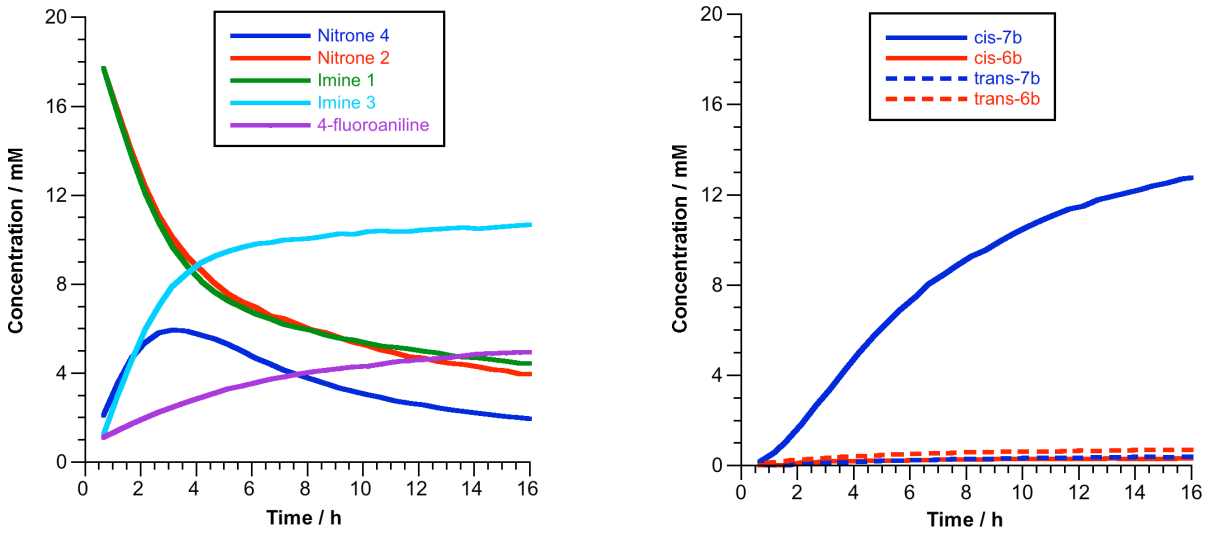


Figure S6 Concentration *vs.* time plot of the exchange process between nitrone **1** and imine **2** in the presence of the recognition-enabled maleimide **5b**. Conditions: [**1**] = [**2**] = [**5b**] = 20 mM; CD_2Cl_2 saturated with PTSA; 273 K. Concentration data derived from 470.4 MHz ^{19}F NMR spectroscopy.